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INVESTIGATIONS IN FISH CONTROL

60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests

61. Toxicity of the Lampricide 3-Trifluoromethlyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests

62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae

United States Department of the Interior
Fish and Wildlife Service

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60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests

    By Leif L. Marking and Lee E. Olson

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62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae

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FOREWORD

The lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), has been used extensively to control larvae of the sea lamprey (Petromyzon marinus) in the Great Lakes. Although the toxicity of TFM to lampreys is well documented, its effects on other organisms are unknown.

The use of any toxicant in the environment raises concern as to the safety of nontarget organisms. Since invertebrate and lower vertebrate populations provide the forage base for many sport and commercial fishes, data on how TFM affects these organisms are vital to any application for registration.

The three papers in this series represent a part of continuing research on the effects of TFM on aquatic organisms. The papers report the results of tests on 15 species of nontarget fish, the larvae of 3 species of frogs, and 16 species of invertebrates. Reports on the effects of TFM on algae, midges, mayflies, and selected other invertebrates were published as Nos.56, 57, 58, and 59 of Investigations in Fish Control; a complete review of the literature prior to 1972 related to the use of TFM as a lampricide was published in No. 44.

Fred P. Meyer, Director
Fish Control Laboratories

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60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests

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TOXICITY OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO NONTARGET FISH IN STATIC TESTS

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ABSTRACT

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is applied to tributary streams of the Great Lakes for controlling larvae of the sea lamprey (Petromyzon marinus). During treatments for lamprey control, cohabiting, nontarget fish are also exposed to TFM. Knowledge of the margin of safety for these fish is vitally important to the reduction of undesired effects of field applications. The lampricide is toxic to 15 species of coldwater and warmwater nontarget fish; the 96-h LC50's in static tests at 12 C range from 1.39 to 16.2μ l/l of field grade TFM (35%). The toxicity of TFM is influenced by temperature, water hardness, and pH. The most influential factor is pH. For certain species, more than 50 times as much chemical is needed to produce the same effect at pH 9.5 as at pH 6.5. In laboratory test water, TFM detoxifies slowly; solutions lose little or no activity over periods up to 8 wk. The margin of safety (LC01 for fish/LC99 for lamprey) for rainbow trout (Salmo gairdneri) in minimum lampricidal concentrations of TFM is influenced by pH and is greater in water of low pH (6.5) than in water of higher pH. Under laboratory conditions at pH 7.5 and 8.5, a 10% mortality of rainbow trout could be expected in lampricidal concentrations of field grade TFM.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is an effective toxicant against larval lampreys (Petromyzon marinus) living in tributary streams of the Great Lakes (Applegate et al. 1958). However, additional data on the toxicity of TFM to nontarget organisms are needed to satisfy regulatory requirements for toxicants (Lennon 1967). Previous laboratory and field information regarding the use of this lampricide was summarized by Schnick (1972).

The present study was designed to determine the toxicity of purified, field grade, and reduced TFM to fish in laboratory toxicity tests and to determine the influence of water hardness, pH, and temperature on the toxicity of TFM. The residual toxicity of TFM in water solutions was determined to evaluate the persistence of the toxicant under aerobic conditions. These data were used to derive the margin of safety for nontarget fish.

MATERIALS AND METHODS

The static test procedures used follow closely those of Lennon and Walker (1964) and Taras (1971). Ten fish were exposed to each concentration of TFM in glass jars containing 15 liters of oxygen-saturated test water. The test waters were prepared according to the schedule in Table 1 to produce desired water hardnesses. In separate studies, the pH of

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Table 1.--Quantities of salts and characteristics of reconstituted waters

Water type	Salts added in mg/l				pH	mg/l as $CaCO_3$	
	$NaHCO_3$	$CaSO_4.2H_2O$	$MgSO_4$	KCl		Hardness	Alkalinity
Very soft	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft	48	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Hard	192	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Very hard	384	240.0	240.0	16.0	8.0-8.4	280-320	225-245

test waters was controlled with chemical buffers (Table 2). The solutions were adjusted to the appropriate pH before the test and readjusted with chemical buffers at 24-h intervals as necessary to maintain the selected pH ± 0.2 units. Test temperatures were regulated by immersing the test jars in constant-temperature water baths.

Table 2.--Buffer chemicals used to produce and maintain various pH's in soft, reconstituted water

pH	Milliliters of solutions for 15 liters of water		
	1N NaOH	1M KH_2PO_4	0.5M H_3BO_3
6.0	1.3	80.0	---
6.5	10.0	30.0	---
7.0	19.0	30.0	---
7.5[a]	---	---	---
8.0	19.0	20.0	---
8.5	12.0	11.5	---
9.0	8.8	---	30.0
9.5	11.0	---	20.0
10.0	16.0	---	18.0

[a]Unbuffered soft, reconstituted water.

Field grade TFM and analytical grade TFM (99% active ingredient) were obtained from Hoescht Chemical Company, Summerville, New Jersey.[2] Field grade TFM is formulated with DMF (N,N-dimethylformamide) and is approximately 35% active ingredient, but the purity varies slightly between batches. Purified TFM was prepared by Aldrich Chemical Company[2] (96% active ingredient). Dr. John Lech of the Department of Pharmacology of the Medical College of Wisconsin at Milwaukee also prepared purified TFM (94% active ingredient) and synthesized reduced TFM (RTFM) hydrochloride for these experiments. The purified materials were weighed on an electrobalance and dissolved in acetone; concentrations are expressed as mg/l. Field-grade TFM was measured volumetrically and dissolved in water; concentrations are expressed as µl/l.

Fish weighing 1 to 1.5 g each were obtained from Federal hatcheries and maintained according to the standard procedures of the Fish Control Laboratory (Hunn et al. 1968). The fish were acclimated to the desired water chemistries and temperature of each test. Mortalities were recorded at 1, 3, and 6 h on the first day of exposure and daily thereafter for the remainder of the 96-h test.

The methods of Litchfield and Wilcoxon (1949) were used in computation of the LC50's (concentrations producing 50% mortality) and

[2]Use of trade names does not imply U.S. Government endorsement of commercial products.

Table 3.--Toxicity of purified TFM[a] to fingerling fish in toxicity tests at 12 C

Species	LC50 and 95% confidence interval (mg/l) at				
	1 h	3 h	6 h	24 h	96 h
Coho salmon (Oncorhynchus kisutch)	6.80 6.24-7.41	5.60 4.69-6.69	5.60 4.69-6.69	4.30 3.78-4.89	2.70 2.26-3.22
Chinook salmon (Oncorhynchus tshawytscha)	4.00 3.30-4.86	3.40 3.06-3.78	---	3.10 2.70-3.55	2.24 1.94-2.59
Rainbow trout (Salmo gairdneri)	4.40 4.02-4.82	3.08 2.87-3.31	2.92 2.65-3.22	2.91 2.57-3.31	1.97 1.78-2.18
Brown trout (Salmo trutta)	7.00 6.33-7.74	4.93 4.47-5.43	4.78 4.20-5.44	3.89 3.57-4.24	2.63 2.35-2.94
Lake trout (Salvelinus namaycush)	2.72 2.30-3.21	1.93 1.71-2.18	1.43 1.16-1.75	1.43 1.16-1.75	1.40 1.11-1.77
Northern pike (Esox lucius)	5.55 4.67-6.59	1.85 1.25-2.73	1.85 1.25-2.73	1.25 0.847-1.84	0.947 0.594-1.51
Carp (Cyprinus carpio)	---	---	2.10 1.85-2.37	1.74 1.43-2.11	1.25 1.00-1.56
Channel catfish (Ictalurus punctatus)	5.15 4.26-6.23	2.38 2.05-2.76	1.34 1.20-1.50	1.20 1.03-1.40	1.00 0.803-1.25
Bluegill (Lepomis macrochirus)	12.9 11.4-14.6	8.90 8.22-9.64	6.42 5.99-6.89	6.23 5.50-7.05	6.23 5.50-7.05
Smallmouth bass (Micropterus dolomieui)	11.1 10.2-12.1	7.96 7.10-8.93	6.42 5.66-7.28	6.42 5.66-7.28	6.30 5.63-7.04
Largemouth bass (Micropterus salmoides)	---	5.45 5.01-5.93	3.85 3.41-4.35	2.19 1.82-2.63	---
Yellow perch (Perca flavescens)	6.20 5.05-7.61	3.38 2.63-4.35	2.88 2.47-3.35	2.51 2.19-2.88	2.07 1.69-2.54
Walleye (Stizostedion vitreum)	7.10 4.96-10.1	3.00 2.47-3.65	2.05 1.84-2.28	1.88 1.61-2.19	1.88 1.63-2.16

[a]Purity (94%) for tests with coho salmon and (96%) for tests with others.

95% confidence intervals. Regressions were drawn and inspected for each set of data. All data fulfilled the Chi-square test requirement for acceptability.

Deactivation indices were derived for field grade TFM in water at four different pH's. Aged solutions of the toxicant were bioassayed to determine the biological activity remaining after selected time periods. The deactivation index was determined by dividing the LC50 of aged solutions by the LC50 of unaged solutions under corresponding test conditions (Marking 1972).

RESULTS

Purified TFM

Toxicity to selected species of fish

Purified TFM is toxic to coldwater and warmwater fish in soft water; the 96-h LC50's range from 0.947 to 6.30 mg/l of TFM (Table 3). Northern pike and channel catfish were most sensitive, and the 96-h LC50's were not significantly different from each other ($P = 0.05$). Smallmouth bass and bluegill are the most resistant; LC50's were significantly different from those with other species at all comparable exposure periods. Most of the other species were sensitive to 1 to 3 mg/l of TFM.

All species responded rapidly to the toxic effects of TFM (as shown by the 1-h LC50's), and the toxicity changed little with prolonged exposure (the 1-h LC50's which are only 2 to 5 times greater than 96-h LC50's). The LC50's for 24- and 96-h exposures were not significantly different for lake trout, northern pike, channel catfish, smallmouth bass, bluegill, yellow perch, and walleye.

Influences of temperature, water hardness, and pH

The toxicity of purified TFM to fish was altered considerably by water quality, and the alteration was fairly uniform for different species. Test results are presented for rainbow trout in Table 4 and for other species in Appendix Tables 1 to 7.

The lampricide was more toxic to rainbow trout in warm than in cold water. The 96-h LC50's were significantly different at temperatures of 7, 12, and 17 C. This influence was more consistent for coldwater species than for warmwater species. The 96-h LC50's for carp, for instance, were not significantly different at 12, 17, and 22 C.

Purified TFM was more toxic to rainbow trout in soft water (total hardness, 44 mg/l) than in hard or very hard water (total hardness, 170 and 300 mg/l, respectively). The respective 96-h LC50's at 12 C were 1.97, 5.47, and 9.45 mg/l. The hardness of test water influenced the toxicity to other species in a similar manner.

The toxicity of purified TFM to fish decreased substantially as the pH of test waters increased (Table 4 and Appendix Tables 1 to 7). The 96-h LC50's were significantly different for each pH increment for rainbow trout as well as for other species. The magnitude of change in toxicity can be compared by dividing the LC50 value at the lowest pH by that at the highest pH. The factors are as follows: coho salmon - 45, rainbow trout - 29, brown trout - 50, lake trout - 59, carp - 36, channel catfish - 23, bluegill - 21, and yellow perch - 26. The factor for rainbow trout is lower than that for other salmonids because the pH ranged only from 6.5 to 9.0, whereas for the other species the pH ranged from 6.5 to 9.5.

Field grade TFM

Toxicity to selected species of fish

Field grade TFM also was toxic to coldwater and warmwater fishes in soft water (Table 5). The 96-h LC50's for 15 species ranged from 1.39 to 16.2μ l/l of TFM (35.4%). Considering only the active ingredient in the formulation, the range was 0.39 to 4.58μ l/l. Field grade TFM thus appeared to be more active than purified TFM; however, the difference was slight and may have been due to variations in sensitivity among the different groups of fish exposed. Among the families of fishes represented, centrarchids were the most resistant to TFM. Bluegill and green

Table 4.--Toxicity of purified TFM (96%) to fingerling rainbow trout at selected temperatures, water hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	6.20 5.61-6.85	4.00 3.73-4.29	3.00 2.78-3.24	2.89 2.64-3.16	2.44 2.16-2.75
12	Soft	7.5	4.40 4.02-4.82	3.08 2.87-3.31	2.92 2.65-3.22	2.91 2.57-3.30	1.97 1.78-2.18
17	Soft	7.5	3.10 2.93-3.28	2.62 2.33-2.94	2.62 2.33-2.94	2.05 1.70-2.47	1.58 1.35-1.85
12	Hard	7.8	---	9.00 8.41-9.63	8.43 7.67-9.26	8.00 7.43-8.61	5.45 4.74-6.27
12	Very hard	8.2	18.5 16.4-20.9	15.0 13.9-16.2	14.7 13.5-16.0	13.8 12.6-15.1	9.45 9.12-9.79
12	Soft	6.5	2.00 1.74-2.30	1.42 1.28-1.57	1.30 1.17-1.45	1.26 1.13-1.39	1.10 0.744-1.63
12	Soft	8.0	15.4 14.3-16.5	10.9 9.96-11.9	10.0 9.39-10.6	7.75 6.82-8.80	6.19 5.56-6.89
12	Soft	9.0	---	---	---	37.9 33.5-42.9	32.1 29.5-34.9

sunfish were the most resistant species to field grade TFM and smallmouth bass to purified TFM. Channel catfish were the most sensitive species to field grade TFM.

Influences of temperature, water hardness, and pH

The toxicity of field grade TFM to fish was influenced by temperature, water hardness, and pH in patterns similar to those observed with purified TFM. In general, TFM (35.7%) was most toxic to fish in warm, very soft, and low pH (6.5) water. Toxicity data for rainbow trout are in Table 6, and those for other species are in Appendix Tables 8-13. The greatest influence on the toxicity of TFM (35.7%) was from pH. Several 96-h LC50's were unavailable, but the 24-h exposure produced a good approximation of the 96-h results. The 24-h toxicity of TFM (35.7%) to rainbow trout decreased by a factor of approximately 10 as pH increased from 6.5 to 8.5 and by a factor of nearly 100 as pH increased from 6.5 to 9.5 These factors were much greater than those for purified TFM at pH's of 6.5 to 9.0 (Table 4). Apparently the toxicity change accelerated above pH 9.0. Also, the data for most other species (Appendix Tables 8-13) showed a significant increase in toxicity from 24- to 96-h exposures at the high pH; the LC50's at pH 9.5 were 31 and 83 times greater than those at pH 6.5 for yellow perch and lake trout, respectively.

Reduced TFM

In waters of three different hardnesses, the reduced form of purified TFM was considerably less toxic to rainbow trout than the parent material. The 96-h LC50's ranged from 29.0 mg/l of RTFM in very soft to 48.0 mg/l in very hard water (Table 7); however, the

Table 5.--Toxicity of field grade TFM (35.4%)[a] to fingerling fish in toxicity tests at 12 C

Species	LC50 and 95% confidence interval (µl/l) at				
	1 h	3 h	6 h	24 h	96 h
Chinook salmon (Oncorhynchus tschawytscha)	11.5 9.66-13.7	8.66 7.86-9.54	7.62 6.48-8.96	5.98 5.09-7.03	4.20 3.52-5.02
Brown trout (Salmo trutta)	9.63 8.46-11.0	5.83 5.33-6.38	4.94 4.15-5.88	4.53 3.86-5.32	3.53 3.04-4.09
Rainbow trout (Salmo gairdneri)	5.83 5.36-6.34	4.83 4.33-5.39	4.46 4.05-4.91	3.83 3.31-4.43	3.83 3.31-4.43
Lake trout (Salvelinus namaycush)	14.5 12.5-16.9	4.94 3.81-6.38	4.52 3.50-5.82	3.84 3.11-4.72	2.94 2.64-3.28
Goldfish (Carassius auratus)	38.5 29.4-50.4	12.7 10.8-15.0	7.17 6.50-7.91	5.22 4.32-6.30	5.00 3.97-6.29
Carp (Cyprinus carpio)	8.27 7.00-9.77	4.51 3.24-6.29	3.35 2.37-4.74	3.35 2.37-4.74	3.35 2.37-4.74
Golden shiner (Notemigonus (crysoleucas)	18.8 18.3-19.3	11.4 9.98-13.0	10.0 9.01-11.1	8.20 7.10-9.48	7.62 6.29-9.23
Fathead minnow (Pimephales promelas)	17.5 15.9-19.3	10.5 8.14-13.5	5.54 4.65-6.60	4.79 4.19-5.47	4.79 4.19-5.47
White sucker (Catostomus commersoni)	10.0 8.24-12.1	6.50 5.16-8.19	6.26 4.94-7.93	4.50 3.22-6.28	3.95 2.69-5.81
Black bullhead (Ictalurus melas)	13.5 12.2-14.9	5.50 4.67-6.48	3.85 3.29-4.50	2.34 1.89-2.90	2.41 2.10-2.77
Channel catfish (Ictalurus punctatus)	11.9 10.2-13.9	4.64 4.09-5.25	3.86 3.30-4.51	2.40 2.08-2.77	1.39 1.12-1.73
Green sunfish (Lepomis cyanellus)	26.2 24.3-28.3	16.8 14.9-18.9	13.1 11.6-14.7	12.9 11.4-14.6	9.40 7.88-11.2
Bluegill (Lepomis macrochirus)	---	25.4 21.5-30.0	16.2 14.1-18.6	16.2 13.6-19.3	16.2 13.6-19.3
Largemouth bass (Micropterus salmoides)	---	---	10.0 5.28-18.9	6.04 4.07-8.97	6.04 4.07-8.97
Yellow perch (Perca flavescens)	11.4 10.0-12.9	7.00 6.35-7.71	5.85 5.27-6.49	5.80 4.98-6.76	4.35 3.45-5.48

[a] TFM (35.7%) for chinook salmon, brown trout, lake trout, and rainbow trout.

Table 6.--Toxicity of field grade TFM (35.7%) to fingerling rainbow trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	10.2 9.16-11.4	6.68 5.93-7.52	4.78 4.23-5.40	4.37 3.95-4.84	3.68 3.38-4.01
12	Soft	7.5	5.83 5.36-6.34	4.83 4.33-5.39	4.46 4.05-4.91	3.83 3.31-4.43	3.83 3.31-4.43
17	Soft	7.5	4.10 3.75-4.48	3.40 3.05-3.79	3.40 3.05-3.79	2.79 2.34-3.33	2.37 2.05-2.75
12	Very soft	6.6	3.77 3.32-4.28	3.27 2.85-3.75	---	---	---
12	Hard	7.8	50.3 43.5-58.2	26.0 23.0-29.4	19.0 16.7-21.6	14.1 12.8-15.5	8.38 7.41-9.48
12	Very hard	8.2	88.3 79.4-98.2	45.9 40.5-52.0	36.6 33.2-40.4	27.2 21.8-34.0	19.0 16.8-21.5
12	Soft	6.5	4.12 3.71-4.57	2.82 2.56-3.10	2.56 2.17-3.01	2.52 2.16-2.94	2.52 2.16-2.94
12	Soft	8.5	74.0 65.0-84.2	42.4 38.5-46.7	36.7 32.1-42.0	20.5 --- ---	---
12	Soft	9.5	>300	270 228-320	239 205-278	230 204-259	---

Table 7.--Toxicity of reduced TFM to fingerling rainbow trout in standard, reconstituted water at 12 C

Water hardness	LC50 and 95% confidence interval (mg/l) at		
	24 h	48 h	96 h
Very soft	30.0 24.6-36.6	30.0 24.6-36.6	29.0 26.2-32.1
Hard	64.0 51.7-79.2	60.0 49.2-73.2	49.0 42.7-56.3
Very hard	52.0 44.4-60.9	50.0 43.3-57.7	48.0 41.5-55.5

influence of water hardness was not as great as with the parent compound.

Residual Toxicity

Field grade TFM (35.4%) was added to test waters and permitted to age for 1 wk before rainbow trout were introduced. A comparison of the toxicity of aged solutions and unaged reference solutions showed that TFM detoxified slowly, if at all, in water solutions (Table 8). The 96-h deactivation index (LC50 of aged solution/LC50 of unaged solution) was 0.91 for pH 6.5, 1.03 for pH 7.5, 1.09 for pH 8.0, and 1.14 for pH 9.0. Although detoxification tended to be slightly greater at high pH's, the toxicity of aged and unaged solutions was not significantly different at any pH's.

Additional deactivation studies were carried out for longer aging periods to determine the rate of detoxification at different pH's. The toxicity and deactivation indices of purified TFM (96%) were determined at four pH's after aging periods up to 8 wk at 12 C (Table 9). The indices were near 1.0 for pH's 6.5, 7.5, and 8.5 but were erratic and did not show significant detoxification of TFM with aging. TFM was much less toxic to rainbow trout at pH 9.5 than at lower pH's. Activity decreased with aging, but the decrease again was erratic. Although the index at 4 wk of aging (1.95) indicated a considerable decrease in activity, the activity remained constant in a series of solutions aged for 6 wk. Apparently, TFM was detoxified in some instances but not in others. Determination of the rate of detoxification

Table 8.--Toxicity of field grade TFM (35.4%) to fingerling rainbow trout in buffered solutions at 12 C freshly prepared (F) or aged 1 week (A)

pH	Type of solution	LC50 and 95% confidence interval (µl/l) at				
		1 h	3 h	6 h	24 h	96 h
6.5	(F)	3.45 3.10-3.84	3.00 2.68-3.35	2.85 2.59-3.13	2.85 2.59-3.13	2.46 2.17-2.79
	(A)	4.00 3.57-4.49	3.30 2.87-3.79	3.29 2.87-3.77	3.10 2.75-3.49	2.24 2.01-2.50
7.5	(F)	11.9 10.3-13.8	8.70 7.98-9.48	8.70 7.98-9.48	6.33 5.63-7.11	4.78 3.97-5.76
	(A)	19.1 16.7-21.9	11.6 9.96-13.5	11.1 9.39-13.1	8.58 7.34-10.0	4.90 3.61-6.66
8.0	(F)	42.0 36.0-49.0	35.0 30.6-40.0	32.0 28.4-36.1	26.0 23.6-28.7	11.0 10.1-12.0
	(A)	42.0 38.4-45.9	33.0 29.3-37.2	31.0 27.6-34.8	23.0 21.0-25.2	12.0 10.4-13.8
9.0	(F)	---	---	---	---	39.5 33.8-46.2
	(A)	238 187-303	142 121-167	137 118-159	117 99.2-138	45.0 38.5-52.5

Table 9.--Toxicity to rainbow trout of fresh and aged solutions of purified TFM (96%) at four pH's in 12 C water. (Deactivation indices shown in parentheses)

Aging period (weeks)	96-h LC50 and 95% confidence interval (mg/l) and (deactivation index)			
	pH 6.5	pH 7.5	pH 8.5	pH 9.5
0	0.962 0.850-1.09 (1.00)	2.08 1.73-2.50 (1.00)	5.39 4.84-6.00 (1.00)	40.5 35.8-45.8 (1.00)
1	---	2.32 1.98-2.71 (1.12)	5.81 5.24-6.45 (1.08)	71.2 64.9-78.2 (1.76)
2	---	2.17 1.83-2.57 (1.04)	5.63 5.09-6.22 (1.04)	66.3 59.2-74.2 (1.64)
3	---	---	4.40 --- (0.816)	---
4	---	1.78 --- (0.856)	---	79.0 69.4-89.9 (1.95)
6	1.20 0.973-1.48 (1.25)	2.05 1.70-2.47 (0.986)	5.81 5.23-6.46 (1.08)	51.8 46.8-57.3 (1.28)
8	0.842 --- (0.875)	2.05 1.71-2.46 (0.986)	---	---

from these erratic indices was not possible nor was it feasible to extend the aging periods. The data were sufficient, however, to indicate that TFM did not detoxify readily under laboratory conditions. Concentrations of TFM remaining in aged solutions were confirmed by spectrophotometric analysis (Olson and Marking 1973).

Comparison of Various Formulations

Some of the various formulations of TFM that have been prepared for laboratory use and for field applications were tested to determine their activity against rainbow trout (Table 10). The high-percentage formulations tested were comparable in activity but were slightly less active than field grade TFM (35.7%). The greater activity of the field grade TFM was expected because the formulating process overcomes some of the problems associated with solubility. The carrier used in the field grade formulation may increase dispersion, reduce particle size, or enhance ionic state of the TFM molecule since may carriers are used for such purposes.

Table 10.--Toxicity of TFM in various formulations to rainbow trout in soft water at 12 C

Percent active ingredient		Toxic unit	96-h LC50 and 95% confidence interval at	
			Total formulation	Active TFM
Analytical	(99+)	mg/l	1.39 1.17-1.66	1.39 1.17-1.66
Purified	(96)	mg/l	1.50 1.35-1.67	1.44 1.27-1.57
Purified	(94)	mg/l	1.55 1.36-1.76	1.46 1.28-1.65
Field	(35.7)	µl/l	3.38 2.91-3.92	1.21 1.03-1.40

DISCUSSION

Data on the toxicity of TFM to nontarget fishes helps assess the margin of safety for such fish. Because the toxicity of TFM is influenced significantly by water hardness and pH, the margin of safety can be determined accurately only if the tests for toxicity to target and nontarget organisms are done in comparable water media. Toxicity data from field applications usually cannot be compared with laboratory toxicity data because of the differences in water quality and the presence of other biota. Ideally, the margin of safety should be determined by testing field grade TFM against target and nontarget organisms in the water to be treated.

Efficacious concentrations of field grade TFM were determined in standardized laboratory tests by Dawson et al. (In press), who used test water identical to that used in our toxicity tests. For example, in soft water (44 mg/l total hardness; 12 C), the 24-h LC99 in µl/l of TFM (35.7%) against larval lamprey was 0.950 at pH 6.5, 3.25 at pH 7.5, and 12.0 at pH 8.5. In corresponding water quality, the 24-h LC01 in µl/l of TFM (35.7%) against rainbow trout was 1.60 at pH 6.5, 2.60 at pH 7.5, and 10.4 at pH 8.5. The margin of safety (LC01 for fish/LC99 for lamprey) at the respective pH's was 1.760, 0.800, and 0.866. Because values less than 1.0 indicate incomplete survival of the trout, some trout would be expected to die at minimum lampricidal concentrations at pH 7.5 or 8.5, but not at pH 6.5.

If the margin of safety is calculated on the basis of LC10's for fish and LC99's for lamprey, the value is near or higher than 1.0 at the three pH's. Therefore, a 10% mortality of rainbow trout could be expected at pH's 7.5 and 8.5 under these conditions. Trout in pH 6.5 water are safe (LC10/LC99 = 2.11).

CONCLUSIONS

1. Purified and field grade TFM are toxic to coldwater and warmwater fish in brief exposures (1, 3, and 6 h) as well as in 96-h exposures. The toxicity increased little during prolonged exposures.
2. TFM was generally more toxic to fish at higher temperatures, but the trend was not consistent for all warmwater species.
3. TFM was more toxic to fish in very soft water than in very hard water by a factor as great as 10.
4. TFM was considerably more toxic in acid than in alkaline water. The factor was more than 50 for pH's 6.5 to 9.5 for some species.

5. Reduced TFM was less toxic than TFM to fish, but the toxicity of RTFM was influenced less by water hardness.

6. TFM was very persistent in laboratory test waters; activity decreases were small or nil for periods up to 8 wk.

7. On the basis of active ingredient, field grade TFM appeared to be slightly more toxic than purified TFM.

8. The margin of safety for rainbow trout in minimum lampricidal concentrations of field grade TFM was influenced by pH and was greatest at pH 6.5.

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APPENDIX

Appendix Table 1.--Toxicity of purified TFM (94%) to fingerling coho salmon at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	9.60 8.74-10.5	6.10 5.37-6.93	5.60 4.69-6.69	4.80 4.20-5.49	4.30 3.73-4.95
12	Soft	7.5	6.80 6.24-7.41	5.60 4.69-6.69	5.60 4.69-6.69	4.30 3.78-4.89	2.70 2.26-3.22
17	Soft	7.5	3.61 3.35-3.89	3.47 3.11-3.87	3.47 3.11-3.87	2.68 2.39-2.99	2.00 1.64-2.43
12	Very soft	6.6	7.40 6.64-8.24	4.60 4.10-5.16	4.55 4.20-4.93	2.90 2.62-3.21	1.70 1.45-1.99
12	Hard	7.8	16.0 14.7-17.5	15.6 14.5-16.8	15.0 13.5-16.7	13.2 12.1-14.4	6.50 5.39-7.83
12	Very hard	8.2	33.0 30.6-35.6	33.0 30.6-35.6	32.0 29.8-34.3	25.4 23.8-27.1	17.0 15.3-18.9
12	Soft	6.5	2.30 2.05-2.58	1.72 1.54-1.92	1.69 1.52-1.88	1.67 1.45-1.92	1.18 1.02-1.36
12	Soft	8.5	18.5 16.2-21.1	16.9 15.2-18.7	16.3 14.2-18.7	11.9 10.2-13.8	8.25 7.18-9.49
12	Soft	9.5	---	---	---	---	51.3 39.4-66.8

Appendix Table 2.--Toxicity of purified TFM (96%) to fingerling brown trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	8.21 7.15-9.43	5.00 4.55-5.49	4.05 3.43-4.79	2.93 2.35-3.65	2.60 2.31-2.93
12	Soft	7.5	7.00 6.33-7.74	4.93 4.47-5.43	4.78 4.20-5.44	3.89 3.57-4.24	2.63 2.35-2.94
17	Soft	7.5	5.61 5.10-6.17	3.80 3.25-4.44	3.80 3.25-4.44	3.58 3.22-3.98	1.80 1.65-1.97
12	Very soft	6.6	3.60 3.17-4.09	2.48 2.18-2.82	2.30 2.08-2.54	2.04 1.87-2.22	1.20 1.02-1.41
12	Hard	7.8	10.8 9.87-11.8	8.41 7.65-9.25	8.00 7.35-8.70	8.00 7.35-8.70	5.80 --- ---
12	Very hard	8.2	16.3 15.0-17.7	11.9 10.4-13.6	11.9 10.4-13.6	11.9 10.4-13.6	9.80 9.25-10.4
12	Soft	6.5	2.37 2.05-2.74	1.33 1.19-1.48	1.30 1.15-1.47	1.12 1.02-1.23	0.990 0.881-1.11
12	Soft	8.5	26.8 21.3-33.7	17.0 15.3-18.9	14.8 12.8-17.2	12.8 11.5-14.2	8.28 7.31-9.37
12	Soft	9.5	>60.0	>60.0	>60.0	>60.0	50.0 47.0-53.2

Appendix Table 3.--Toxicity of purified TFM (96%) to fingerling lake trout at selected temperatures, water hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	6.02 4.89-7.41	2.23 1.83-2.71	1.89 1.61-2.22	1.74 1.55-1.95	1.62 1.41-1.86
12	Soft	7.5	2.72 2.30-3.21	1.93 1.71-2.18	1.43 1.16-1.75	1.43 1.16-1.75	1.40 1.11-1.77
12	Very soft	6.6	2.35 2.13-2.59	1.35 1.21-1.51	1.19 1.02-1.38	1.19 1.02-1.38	1.07 0.850-1.35
12	Hard	7.8	10.6 9.43-11.9	6.68 6.14-7.27	5.93 5.45-6.45	5.85 5.31-6.45	4.05 3.49-4.70
12	Very hard	8.2	18.0 16.5-19.7	12.2 11.6-12.9	11.1 10.4-11.8	10.7 9.88-11.6	8.42 7.64-9.28
12	Soft	6.5	1.81 1.61-2.04	0.842 0.750-0.945	0.704 0.593-0.836	0.704 0.593-0.836	0.690 0.569-0.837
12	Soft	8.5	---	9.42 8.04-11.0	8.52 7.73-9.39	7.75 7.06-8.51	4.58 3.48-6.03
12	Soft	9.5	52.0 39.5-68.4	50.0 38.8-64.5	50.0 38.8-64.5	50.0 38.8-64.5	40.8 34.5-48.3

Appendix Table 4.--Toxicity of purified TFM (96%) to fingerling carp at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	---	---	2.10 1.85-2.37	1.74 1.43-2.11	1.25 1.00-1.56
17	Soft	7.5	4.30 3.77-4.90	2.52 2.30-2.77	2.36 2.08-2.67	2.36 2.08-2.67	1.42 1.19-1.70
22	Soft	7.5	4.27 3.73-4.88	2.93 2.51-3.42	2.82 2.44-3.26	2.33 2.01-2.71	1.39 1.14-1.70
12	Very soft	6.6	3.46 3.22-3.72	1.87 1.65-2.12	1.46 1.32-1.62	1.28 1.06-1.54	1.03 0.793-1.34
12	Hard	7.8	13.8 12.2-15.6	7.75 7.20-8.34	6.00 5.35-6.73	5.37 4.69-6.14	3.63 3.08-4.28
12	Very hard	8.2	19.0 17.6-20.5	10.3 9.38-11.3	7.72 7.20-8.28	6.00 5.00-7.20	4.62 4.03-5.30
12	Soft	6.5	2.05 1.87-2.24	---	0.820 0.715-0.940	0.770 0.602-0.984	0.770 0.602-0.984
12	Soft	8.5	---	9.00 8.36-9.69	8.10 7.08-9.27	4.46 3.66-5.42	3.15 2.34-4.22
12	Soft	9.5	---	43.9 40.6-47.3	42.6 38.5-47.2	35.7 32.2-39.6	28.0 23.6-33.3

Appendix Table 5.--Toxicity of purified TFM (96%) to fingerling channel catfish at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	5.05 4.18-6.10	2.60 1.99-3.40	1.18 1.02-1.36	1.12 0.945-1.33	0.905 0.838-0.978
17	Soft	7.5	2.37 2.05-2.74	1.53 1.35-1.73	1.06 0.960-1.17	0.970 0.859-1.10	0.970 0.859-1.10
22	Soft	7.5	2.16 1.59-2.94	1.18 1.01-1.37	0.882 0.786-0.990	0.765 0.712-0.822	0.765 0.712-0.822
12	Very soft	6.6	> 3.00	2.43 2.03-2.90	0.775 0.688-0.872	0.478 0.431-0.530	0.478 0.431-0.530
12	Hard	7.8	12.1 9.93-14.7	7.25 6.15-8.55	3.51 3.14-3.93	2.59 2.29-2.93	2.59 2.29-2.93
12	Very hard	8.2	25.5 22.0-29.6	8.90 7.95-9.96	7.40 6.45-8.50	5.39 4.82-6.02	5.39 4.82-6.02
12	Soft	6.5	> 2.00	1.18 1.02-1.36	0.763 0.706-0.824	0.632 0.584-0.684	0.632 0.584-0.684
12	Soft	8.5	19.0 14.8-24.3	9.37 8.21-10.7	6.43 6.00-6.90	4.52 4.15-4.93	3.38 2.92-3.91
12	Soft	9.5	> 60.0	71.0 52.7-95.6	50.0 43.7-57.2	---	14.4 13.1-15.8

Appendix Table 6.--Toxicity of purified TFM (96%) to fingerling bluegill at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	12.9 11.4-14.6	8.90 8.22-9.64	6.42 5.99-6.89	6.23 5.50-7.05	6.23 5.50-7.05
17	Soft	7.5	7.65 7.09-8.25	5.21 4.64-5.84	4.69 4.39-5.01	4.69 4.39-5.01	3.81 3.22-4.51
22	Soft	7.5	5.50 4.90-6.17	4.65 4.34-4.98	4.65 4.34-4.98	4.65 4.34-4.98	2.19 1.87-2.56
12	Very soft	6.6	7.18 6.54-7.88	---	3.33 2.96-3.75	3.00 2.77-3.25	2.92 2.61-3.27
12	Hard	7.8	22.9 20.4-25.7	---	11.9 10.9-13.0	11.6 10.5-12.8	11.6 10.5-12.8
12	Very hard	8.2	34.0 30.6-37.8	---	23.4 21.0-26.1	23.4 21.0-26.1	23.4 21.0-26.1
12	Soft	6.5	7.80 7.16-8.50	3.40 3.06-3.78	3.40 3.06-3.78	3.39 3.05-3.77	3.07 2.71-3.48
12	Soft	8.5	33.8 30.5-37.5	24.0 22.6-25.5	24.0 22.6-25.5	22.3 20.6-24.1	22.3 20.6-24.1
12	Soft	9.5	96.3 84.5-110	89.0 82.2-96.4	76.2 70.8-82.0	76.2 70.8-82.0	64.2 60.0-68.7

Appendix Table 7.--Toxicity of purified TFM (96%) to fingerling yellow perch at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	6.20 5.05-7.61	3.38 2.63-4.35	2.88 2.47-3.35	2.51 2.19-2.88	2.07 1.69-2.54
17	Soft	7.5	4.77 4.18-5.45	2.68 2.22-3.24	2.68 2.22-3.24	2.13 1.92-2.36	2.05 1.67-2.51
22	Soft	7.5	3.38 3.05-3.75	2.80 2.25-3.49	2.80 2.25-3.49	2.46 2.17-2.79	1.78 1.60-1.98
12	Very soft	6.6	---	---	---	1.68 1.24-2.28	1.22 0.932-1.60
12	Hard	7.8	---	12.0 10.5-13.7	9.00 7.55-10.7	7.00 5.91-8.30	6.28 5.24-7.53
12	Very hard	8.2	30.0 26.0-34.6	19.5 16.0-22.9	15.2 13.2-17.5	14.2 12.1-16.6	12.2 10.7-13.9
12	Soft	6.5	4.48 3.57-5.62	1.63 1.14-2.33	1.18 1.01-1.37	1.10 0.930-1.30	1.00 0.870-1.15
12	Soft	8.5	22.2 17.7-27.8	12.0 10.1-14.3	10.4 8.82-12.3	9.42 8.14-10.9	8.80 7.53-10.3
12	Soft	9.5	47.2 39.2-56.8	44.3 38.3-51.3	44.3 38.3-51.3	44.2 35.7-54.8	26.2 21.7-31.7

Appendix Table 8.--Toxicity of field grade TFM (35.7%) to fingerling chinook salmon at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	15.9 13.0-19.4	8.47 7.70-9.31	6.78 6.12-7.51	6.22 5.51-7.03	4.27 3.89-4.69
12	Soft	7.5	11.5 9.66-13.7	8.66 7.86-9.54	7.62 6.48-8.96	5.98 5.09-7.03	4.20 3.52-5.02
17	Soft	7.5	8.83 7.91-9.85	7.58 6.66-8.63	6.85 6.16-7.62	5.38 4.83-5.99	3.54 3.19-3.92
12	Very soft	6.6	6.80 6.10-7.59	4.58 4.08-5.15	4.45 4.00-4.95	3.68 3.20-4.23	1.94 1.62-2.33
12	Hard	7.8	38.5 33.9-43.7	28.2 25.7-31.0	22.3 20.0-24.9	16.0 15.2-18.7	10.0 8.98-11.1
12	Very hard	8.2	95.0 80.3-112	60.5 53.5-68.5	58.0 51.2-65.8	41.5 36.0-47.8	19.0 16.2-22.2
12	Soft	6.5	4.70 3.97-5.56	3.58 3.19-4.02	3.49 3.13-3.90	3.12 2.77-3.52	2.45 2.16-2.77
12	Soft	8.5	73.5 67.4-80.1	39.5 35.2-44.4	36.0 31.1-41.6	33.4 28.9-38.6	18.0 15.4-21.0
12	Soft	9.5	> 300	> 300	362 284-462	254 215-300	171 154-190

Appendix Table 9.--Toxicity of field grade TFM (35.7%) to fingerling brown trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at 1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	15.8 13.6-18.4	9.96 8.85-11.2	5.58 5.13-6.07	5.18 4.60-5.83	3.98 3.47-4.56
12	Soft	7.5	9.63 8.46-11.0	5.83 5.33-6.38	4.94 4.15-5.88	4.53 3.86-5.32	3.53 3.04-4.09
17	Soft	7.5	6.53 5.68-7.51	4.72 4.17-5.34	4.72 4.17-5.34	3.82 3.22-4.53	2.14 1.71-2.68
12	Very soft	6.6	7.00 5.98-8.20	3.58 3.15-4.06	3.42 3.07-3.81	3.00 2.48-3.63	2.32 2.08-2.59
12	Hard	7.8	28.8 25.0-33.2	13.8 11.4-16.7	12.8 10.9-15.0	11.2 10.2-12.3	7.22 6.19-8.42
12	Very hard	8.2	59.8 55.2-64.8	39.8 34.7-45.6	35.7 32.5-39.3	22.6 18.7-27.3	18.3 15.1-22.1
12	Soft	6.5	4.10 3.68-4.57	2.46 2.17-2.79	---	2.22 1.99-2.48	2.11 1.91-2.33
12	Soft	8.5	80.0 68.9-92.9	38.8 34.0-44.3	---	28.9 23.9-35.0	25.5 21.5-30.2
12	Soft	9.5	> 150	> 150	> 150	> 150	120 113-127

Appendix Table 10.--Toxicity of field grade TFM (35.7%) to fingerling lake trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence intervals (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	28.2 23.6-33.7	14.0 11.1-17.7	9.63 8.51-10.9	7.28 6.34-8.36	6.18 5.25-7.28
12	Soft	7.5	18.2 14.9-22.2	9.20 8.18-10.4	8.00 7.27-8.80	7.78 7.16-8.46	7.18 6.64-7.77
17	Soft	7.5	>10.0	7.03 5.58-8.86	7.03 5.58-8.86	7.03 5.58-8.86	6.78 5.58-8.23
12	Very soft	6.6	>15.0	10.9 9.98-11.9	7.21 6.57-7.91	4.45 4.06-4.88	3.36 3.01-3.76
12	Hard	7.8	60.3 50.0-73.3	29.2 26.7-31.9	27.3 24.6-30.3	24.7 21.4-28.5	17.2 15.8-18.8
12	Very hard	8.2	92.3 80.5-106	54.2 50.9-57.7	54.0 49.9-58.4	48.3 45.5-51.2	36.2 33.2-39.5
12	Soft	6.5	4.58 4.22-4.97	2.60 2.30-2.94	2.45 2.16-2.77	2.45 2.16-2.77	2.45 2.16-2.77
12	Soft	8.5	>90.0	>90.0	117 89.6-153	76.0 65.7-87.9	45.6 36.4-57.1
12	Soft	9.5	> 300	> 300	> 300	300 267-338	203 180-229

Appendix Table 11.--Toxicity of field grade TFM (35.7%) to fingerling carp at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	13.1 11.6-14.8	6.62[a] 6.10-7.19	6.28 5.55-7.11	5.60 4.65-6.74	5.23 4.37-6.26
17	Soft	7.5	12.2 10.8-13.8	9.60[a] 8.40-11.0	9.58 8.37-11.0	8.20 7.11-9.45	5.42 4.71-6.23
22	Soft	7.5	15.0 14.0-16.1	10.7[a] 9.06-12.6	10.7 9.06-12.6	6.03 4.96-7.33	5.09 4.32-5.99
12	Very soft	6.6	5.99 5.41-6.64	3.58 3.27-3.92	2.68 2.41-2.98	2.52 2.14-2.96	2.52 2.14-2.96
12	Hard	7.8	43.2 34.1-54.7	24.3 20.4-29.0	20.6 18.6-22.9	17.7 16.0-19.6	13.8 12.7-15.0
12	Very hard	8.2	78.5 68.6-89.8	47.2 46.5-47.9	36.0 32.2-40.3	26.9 22.1-32.8	22.3 18.9-26.3
12	Soft	6.5	5.62 5.14-6.15	2.90 2.60-3.24	2.24 1.86-2.69	2.12 1.91-2.35	1.98 1.66-2.36
12	Soft	8.5	84.2 75.0-94.5	38.4 32.2-45.8	29.1 26.2-32.3	16.7 13.9-20.0	14.5 12.9-16.3
12	Soft	9.5	> 300	> 300	279 223-350	169 145-197	96.0 75.2-123

[a]4-h observation.

Appendix Table 12.--Toxicity of field grade TFM (35.7%) to fingerling bluegill at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	44.4 41.2-47.9	19.8 17.2-22.7	15.6 13.6-17.9	13.7 11.7-16.1	13.0 11.5-14.7
17	Soft	7.5	34.0 30.7-37.7	17.9 16.0-20.0	16.0 14.0-18.3	14.6 13.4-15.9	14.6 13.4-15.9
22	Soft	7.5	20.5 18.4-22.8	16.9 15.2-18.7	---	15.5 13.6-17.6	15.0 13.4-16.8
12	Very soft	6.6	30.0 26.8-33.6	11.7 10.2-13.4	8.58 7.73-9.52	7.63 7.08-8.23	7.35 6.74-8.01
12	Hard	7.8	> 120	60.8 53.1-69.7	51.0 46.9-55.5	44.6 40.1-50.0	36.8 33.9-40.0
12	Very hard	8.2	> 200	120 111-130	92.0 84.5-100	71.8 66.4-77.7	67.0 62.2-72.2
12	Soft	6.5	16.9 15.2-18.8	10.5 9.46-11.5	6.23 5.51-7.04	5.62 5.09-6.20	5.62 5.09-6.20
12	Soft	8.5	> 200	153 142-165	98.0 86.0-112	66.5 60.8-72.8	65.4 58.5-73.1
12	Soft	9.5	>400	> 400	478 400-572	368 320-423	368 320-423

Appendix Table 13.--Toxicity of field grade TFM (35.7%) to fingerling yellow perch at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	14.3 11.3-18.1	5.98 5.05-7.09	5.85 4.85-7.05	4.12 3.74-4.54	3.60 2.97-4.37
17	Soft	7.5	9.35 8.31-10.5	5.30 4.76-5.90	5.30 4.76-5.90	4.74 4.18-5.37	4.18 3.63-4.81
22	Soft	7.5	8.30 6.89-10.0	6.00 5.36-6.72	6.00 5.36-6.72	4.05 3.35-4.90	3.76 3.19-4.43
12	Very soft	6.6	6.30 5.60-7.08	2.88 2.26-3.68	2.60 2.22-3.05	2.28 1.92-2.70	2.28 1.92-2.70
12	Hard	7.8	45.0 39.1-51.9	17.2 15.0-19.8	16.9 14.5-19.7	13.4 12.0-15.0	10.3 9.32-11.4
12	Very hard	8.2	93.0 81.4-106	47.6 42.8-53.0	33.3 30.4-36.4	30.4 27.3-33.8	27.0 24.8-29.4
12	Soft	6.5	2.68 2.40-3.00	1.70 1.45-1.99	1.25 0.962-1.62	1.06 0.859-1.31	1.00 0.827-1.21
12	Soft	8.5	42.4 38.3-46.9	20.0 17.3-23.2	13.8 10.3-18.4	9.30 8.19-10.6	5.55 4.44-6.93
12	Soft	9.5	>200	165 147-186	109 92.5-128	82.5 71.8-94.9	31.2 26.8-36.3

61. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests

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Fish and Wildlife Service
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TOXICITY OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO NONTARGET FISH IN FLOW-THROUGH TESTS

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ABSTRACT

Field grade 3-trifluoromethyl-4-nitrophenol (TFM) was tested for acute and chronic toxicity to 11 species of nontarget fish in 4- and 30-day exposures, respectively. The species used were coho salmon (Oncorhynchus kisutch), rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), brook trout (Salvelinus fontinalis), lake trout (Salvelinus namaycush), goldfish (Carassius auratus), golden shiner (Notemigonus crysoleucas), channel catfish (Ictalurus punctatus), bluegill (Lepomis macrochirus), red-ear sunfish (Lepomis microlophus), and yellow perch (Perca flavescens). The 96-h LC50's for the lampricide in flow-through tests ranged from 8.79 to 32.1 μl/l in hard water and from 2.15 to 17.5 μl/l in soft water. The toxicity of the TFM formulation to two species of salmonids did not change significantly ($P = 0.05$) between 1- and 30-day exposures. The results of simultaneous static and flow-through acute toxicity tests with channel catfish were not significantly different in two experiments.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is effective for killing larval lampreys (Petromyzon marinus) living in tributary streams of the Great Lakes without decimating the endemic fish populations (Applegate et al. 1958). The effects of TFM on fish have been observed during numerous stream applications and in the laboratory (Marking and Olson 1975, Dawson et al. (In press), and Schnick 1972). The registration of TFM as a lampricide has been supported primarily by laboratory data developed in static test systems. However, flow-through toxicity tests simulate the use pattern of TFM more closely than static toxicity tests.

This study was designed to determine acute and chronic toxicities of field grade TFM to nontarget fish in flow-through toxicity tests. In addition, the acute toxicity of TFM was compared in static and flow-through systems.

MATERIALS AND METHODS

Field grade TFM, obtained from American Hoechst Chemical Company, Somerville, New Jersey,[1] was used for these experiments. Because the percentage of active ingredient varies from one batch to another, purity is specified.

[1] Use of trade names does not imply U.S. Government endorsement of commercial products.

The liquid formulations were measured volumetrically and diluted in water to prepare stock solutions, and toxicity values were calculated and reported on a formulation volume to volume (μl/l) basis. The concentration of TFM in each test aquarium was determined daily by colorimetric analysis (Olson and Marking 1973), and the toxicity was calculated on the basis of the mean values for the concentrations.

The flow-through toxicity tests were conducted in an apparatus similar to that described by Mount and Brungs (1967) but with modifications according to McAllister et al. (1972). The apparatus was designed to deliver 1 liter of test solution each cycle. Each glass aquarium contained 45 liters of test medium. The rate of flow was sufficient to replace the entire volume of test medium at least four times each day. The flow-through units were designed to deliver seven successively lower concentrations of the toxicant; each concentration was approximately 25% less than the preceding one. The control for each test contained dilution water but no toxicant. The temperature of test solutions was maintained with a water bath.

Two types of water were used in the flow-through tests. Reconstituted water, prepared according to Marking (1969), was used for some 96-h tests and for comparing the toxicity of TFM in static and flow-through tests. Charcoal filtered municipal well water was used for other 96-h tests and for 30-day exposures. The reconstituted water was soft (total hardness of 44 mg/l and pH of 7.5), whereas the well water was hard (total hardness of 300 mg/l and pH of 7.7). Procedures for the static tests followed those of Lennon and Walker (1964).

National Fish Hatcheries furnished the fish for these experiments. Fish used in 96-h tests were not fed during acclimation before each test nor during exposure (Hunn et al. 1968). Fish for the 30-day tests were fed dry commercial pellets during acclimation and exposure. The fish ranged in size from 1.1 to 19.9 g; for tests in which the weight is not specified, the fish weighed 2 to 5 g. Observations on survival and mortality were recorded daily, and dead fish were removed during each observation.

The toxicity of TFM was calculated according to the statistical procedures of Litchfield and Wilcoxon (1949). Toxicity was defined by LC50's (concentrations calculated to produce 50% mortality) and 95% confidence intervals. Chi-square tests were applied to each set of data to test for goodness of fit.

RESULTS

Four species of fish were exposed to field grade TFM (39.45%) in flow-through toxicity tests using soft, reconstituted water at 17 $\pm$ 1 C (Table 1). Channel catfish are the most sensitive and red-ear sunfish the most resistant; the 96-h LC50's were 2.15 and 17.5 μl/l of TFM, respectively. The toxicity of the lampricide did not change significantly ($P = 0.05$) after 24 h for goldfish, golden shiner, and red-ear sunfish.

Seven species were exposed to field grade TFM (35.7%) in charcoal filtered municipal well water at 12 C (Table 2). The 96-h LC50's ranged from 8.79 to 32.1 μl/l of the formulation. Larger fish of a given species were more resistant than smaller ones to the toxicant. For instance, the 96-h LC50 for TFM was 10.5 μl/l against 1.3-g coho salmon and 29.0 μl/l against 7.4-g coho salmon and was 8.79 μl/l against 1.3-g rainbow trout and 13.8 μl/l against 19.7-g rainbow trout. Lake trout (17.0 g) were more resistant than rainbow trout of similar size (96-h LC50 = 16.9 μl/l).

Toxicosis was apparent in very short exposures (1 to 6 h) to TFM, and the toxicity did

Table 1. Toxicity of TFM (39.45%) to fingerling fish in flow-through tests with soft, reconstituted water at 17 ± 1 C

Species	LC50 and 95% confidence interval (µl/l) at			
	3 h	6 h	24 h	96 h
Goldfish (Carassius auratus)	---	8.10 5.65-11.6	4.85 3.26-7.01	4.25 2.86-6.31
Golden shiner (Notemigonus crysoleucas)	---	13.2 9.96-17.5	10.6 8.56-13.1	8.50 5.79-12.5
Channel catfish (Ictalurus punctatus)	7.00 5.22-9.38	4.80 3.82-6.03	4.05 3.11-5.27	2.15 1.52-3.03
Red-ear sunfish (Lepomis microlophus)	30.0 25.5-35.3	---	17.5 14.2-21.6	17.5 14.2-21.6

not change significantly for many exposure time increments (Table 2). In fact, toxicity did not change significantly between 6- and 96-h exposures for coho salmon (7.4 g), rainbow trout, brown trout, brook trout, lake trout, and bluegill. Considering only small sizes of fish, brook trout were more resistant than other salmonids, bluegills, or yellow perch.

Three species of fish were exposed to field grade TFM (35.7%) for 30 days in charcoal filtered municipal well water at 12 C (Table 3). The 30-day LC50's against coho salmon, brook trout, and lake trout ranged from 10.5 to 19.6 µl/l of the formulation. As in shorter exposures, brook trout were most resistant even though they were smaller than lake trout. Also, toxicity did not change significantly between 10 and 30 days with coho salmon and between 1 and 30 days with lake trout.

Channel catfish were exposed to TFM in simultaneous static and flow-through tests to compare the toxicity and to assess the need for establishing the toxicity of TFM in flow-through facilities. Three separate tests showed that TFM (39.45%) was uniformly toxic in the two types of tests (Table 4). All three tests showed that TFM was more toxic in static than in flow-through facilities; however, the difference was significant only in the second trial. Therefore, additional tests in the flow-through facility with water of different temperature, hardness, and pH are perhaps unnecessary because those characteristics have been examined intensively in previous work.

Table 2. Toxicity of TFM (35.7%) to selected fish in 4-day flow-through tests using charcoal filtered municipal well water at 12 C

Species	Average weight (g)	LC50 and 95% confidence interval (µl/l) at				
		1 h	3 h	6 h	24 h	96 h
Coho salmon (Oncorhynchus kisutch)	1.3	14.4 13.2-15.7	14.2 13.2-15.7	14.2 13.2-15.7	12.6 11.3-14.1	10.5 9.21-12.0
Coho salmon	7.4	34.9 28.8-42.3	29.9 25.5-35.0	29.8 25.1-35.3	29.8 25.1-35.3	29.0 25.2-33.4
Rainbow trout (Salmo gairdneri)	1.3	22.0 13.1-36.8	19.7 12.6-30.8	13.2 7.70-22.6	11.4 8.70-14.9	8.79 7.28-10.6
Rainbow trout	19.7	14.8 13.2-16.6	13.8 12.8-14.9	13.8 12.8-14.9	13.8 12.8-14.9	13.8 12.8-14.9
Brown trout (Salmo trutta)	2.7	12.9 12.1-13.7	10.8 10.0-11.7	10.8 10.0-11.7	10.8 10.0-11.7	10.8 10.0-11.7
Brook trout (Salvelinus fontinalis)	2.2	35.4 30.6-41.0	32.1 28.1-36.7	32.1 28.1-36.7	32.1 28.1-36.7	32.1 28.1-36.7
Lake trout (Salvelinus namaycush)	17.0	22.3 18.7-26.6	16.9 15.3-18.7	16.9 15.3-18.7	16.9 15.3-18.7	16.9 15.3-18.7
Bluegill (Lepomis macrochirus)	1.1	---	22.2 19.3-25.2	16.1 14.9-17.4	16.0 14.8-17.3	15.9 14.9-17.0
Yellow perch (Perca flavescens)	2.6	20.6 15.7-27.1	17.6 13.9-22.3	15.1 11.8-19.3	13.2 11.2-15.6	9.44 8.06-11.0

Table 3. Toxicity of TFM (35.7%) to fish in 30-day flow-through tests using charcoal filtered municipal well water at 12 C

Species	Average weight (g)	LC50 and 95% confidence interval (µl/l) at			
		1 day	10 days	20 days	30 days
Coho salmon	1.3	12.6 11.3-14.1	10.5 9.30-11.7	10.5 9.30-11.7	10.5 9.30-11.7
Brook trout	2.2	32.1 28.1-36.7	32.1 28.8-35.8	22.5 19.0-26.6	19.6 15.4-24.9
Lake trout	17.0	16.9 15.3-18.7	16.9 15.3-18.7	16.9 15.3-18.7	16.9 15.3-18.7

Table 4. Toxicity of TFM (39.45%) to channel catfish in static and flow-through tests with soft, reconstituted water at 17 ± 1 C

Type of assay	LC50 and 95% confidence interval (µl/l) at				
	1 h	3 h	6 h	24 h	96 h
Static	14.2 13.3-15.2	6.00 5.39-6.68	4.25 3.89-4.65	3.55 3.16-3.99	2.85 2.45-3.32
Flow-through	---	---	---	3.50 3.17-3.87	3.28 2.99-3.60
Static	12.5 11.6-13.5	6.25 5.53-7.06	3.75 3.07-4.58	3.15 2.72-3.64	2.75 2.29-3.30
Flow-through	---	---	4.85 4.57-5.14	4.30 3.88-4.77	3.42 3.05-3.84
Static	11.8 10.4-13.3	6.60 5.76-7.56	4.60 4.01-5.28	3.48 2.94-4.12	1.80 1.26-2.58
Flow-through	---	7.00 5.22-9.38	4.80 3.82-6.03	4.05 3.11-5.27	2.15 1.52-3.03

DISCUSSION

The use of the flow-through technique has been recommended over the static technique for certain kinds of toxicity determinations. The flow-through technique is more complicated and expensive than the static technique; however, and the use of water with different characteristics is not as practical in flow-through tests as in static tests. Because the toxicity of field grade TFM is similar in both techniques, the static procedure probably is sufficient to estimate the acute toxicity of TFM to fish. However, the flow-through technique must be used for determining chronic toxicity.

Large fish of a species were more resistant than smaller ones. The increase in resistance with size is perhaps related to the greater ability of larger fish to metabolize TFM. Lech and Costrini (1972) demonstrated the formation of TFM glucuronide (reduced TFM) in vitro and suspected that the same metabolite was formed in vivo in rainbow trout. Other studies showed that TFM and reduced TFM are excreted in the urine of rats (Lech 1971). Thus TFM is apparently readily metabolized and excreted. Mature fish probably have more effective enzyme systems than do juveniles for metabolizing TFM and adjusting to a continuous exposure to the toxicant.

The lampricide kills fish in short exposures (1 to 6 h) at concentrations equal to or nearly equal to those required in long exposures (4 to 30 days). In fact, the toxicity did not change after 3 h with salmon (7.4 g), rainbow trout (19.7 g), brown trout, brook trout, and lake trout in 4-day tests (Table 2). The same trend occurred in the 30-day trials in which the LC50's were identical for lake trout after 1 and 30 days of exposure (Table 3). The change in toxicity of TFM to brook trout in 1- and 30-day exposures was significant. However, considering the magnitude of change for brook trout and tests with other species, fish generally succumb immediately or survive chronic exposure by employing enzymatic defenses.

CONCLUSIONS

1. Field grade TFM was toxic to nontarget coldwater and warmwater fish in brief exposures (1 to 6 h) as well as in 96-h exposures in flow-through tests.

2. In hard water, the 96-h LC50's ranged from 8.79 to 32.1 μl/l of TFM formulation and in soft water from 2.15 to 17.5 μl/l of TFM formulation.

3. Field grade TFM was chronically toxic to nontarget fish; however, the toxicity changed little between 1- and 30-day exposures.

4. Field grade TFM was more toxic to small than to large sizes of fish of the same species in 96-h exposures.

5. The toxicity of TFM to fish was greater in static tests than in flow-through tests, but the difference was not significant in two of three experiments.

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INVESTIGATIONS IN FISH CONTROL

62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae

By Jack H. Chandler and Leif L. Marking

United States Department of the Interior
Fish and Wildlife Service
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TOXICITY OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO SELECTED AQUATIC INVERTEBRATES AND FROG LARVAE

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ABSTRACT

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) was tested against various groups of nontarget aquatic organisms. Invertebrates exposed were flatworms (Catenula sp.), annelids (Tubifex tubifex), daphnids (Daphnia magna), seed shrimps (Cypridopsis sp.), glass shrimp (Palaemonetes kadiakensis), mayfly nymphs (Callibaetis sp.), backswimmers (Notonecta sp.), mosquito larvae (Culex sp. and Anopheles sp.), bivalve mollusks (Corbicula sp., Sphaerium sp., Elliptio sp., and Plectomerus sp.), and snails (Physa sp., Helisoma sp., and Pleurocera sp.). Vertebrates exposed to TFM were larvae of gray tree frog (Hyla versicolor), leopard frog (Rana pipiens), and bullfrog (Rana catesbeiana). Larvae of tree frogs were the most sensitive organism to TFM (96-h LC50 = 1.98 mg/l), and backswimmers were the least sensitive (96-h LC50 = 555 mg/l). Soft-bodied invertebrates were less sensitive than snails and bivalve mollusks to TFM. The invertebrates tested were not as susceptible as larval lampreys (Petromyzon marinus) in similar standardized tests.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been effective for controlling sea lamprey (Petromyzon marinus) in the Great Lakes. The lampricide is applied to streams in which the larvae live and is more toxic to larval lampreys than to other fishes (Applegate et al. 1958). Schnick (1972) reviewed the literature on the lampricide and summarized the data available that support existing registration of this pesticide. Recently completed studies have defined the toxicity of TFM to selected nontarget organisms (Marking and Olson 1975; Marking et al. 1974; Maki et al. 1974; Fremling 1974; Kawatski et al. 1974; Sanders and Walsh 1974) and the efficacy against larval lampreys (Dawson et al. In press).

The present study was designed to determine the toxicity of TFM to selected aquatic invertebrates and larvae of frogs.

MATERIALS AND METHODS

Field grade TFM (39.45% active ingredient) was measured gravimetrically and diluted with deionized water to prepare stock solutions for static and flow-through toxicity tests. Concentrations were calculated on the basis of the formulation used in the field rather than on active ingredient.

Static tests were conducted in small jars containing 3 liters of test water or in large jars containing 15 liters of water. The jars were immersed in a water bath to the level of the test fluids; the water bath was equipped with a commercial chilling device (Frigid Units, Inc.[1]). At least 10 concentrations and 1 water control were employed in each test.

Flow-through tests were performed in a modified version of the Mount and Brungs (1967) apparatus, but a different chemical metering device (Chandler et al. 1974) was substituted for the conventional form. Five concentrations and a control were used in each of the flow-through systems. Each of the 5 test chambers held 45 liters of diluted toxicant in which each successive concentration was approximately 50% less than the previous one. The colorimetric method of Olson and Marking (1973) was used periodically to determine actual concentrations of toxicant in each of the aquaria. A rate of flow was maintained which ensured a minimum of three complete replacements of test solutions per day in each of the chambers. Test media were cooled with water bath equipment similar to that used in static tests. Tests were conducted at 16 to 17 C.

Most of the tests were conducted in spring water to which lime was added (hereafter called limed water) to bring the total hardness (as $CaCO_3$) to approximately 20 mg/l. The pH of the test waters varied from 6.8 to 7.0. Reconstituted waters routinely used for toxicity tests involving fish (Marking 1969) were used only in tests with clams, because it appeared in initial tests that the soft-bodied invertebrates might have been adversely affected by the test media.

Most test organisms were collected in ponds and streams. A few were reared outdoors in partly shaded, vinyl pools or in the laboratory. All forms collected in the field were retained for a minimum of 7 days in waters identical with those used in the tests. Only vigorous individuals of uniform sizes were used in tests. Small or delicate organisms were placed in cylindrical cages fabricated from Nitex screen. The cages were suspended in the test chamber of the flow-through apparatus to facilitate observation and to prevent loss or damage to organisms by turbulent water.

Mortality determinations were made on an appropriate hourly or daily basis, and dead organisms and detritus were removed after each examination. Mortalities were based on immobility or lack of response of test organisms to various mechanical stimuli. Snails were assumed to be dead when they failed to retract the "foot" into the shell, and bivalves when they were unable to close their shells.

The statistical procedures of Litchfield and Wilcoxon (1949) were used to calculate the concentration of toxicant necessary to produce 50% mortality (LC50's) and to obtain 95% confidence intervals.

RESULTS AND DISCUSSION

Eight groups of invertebrates (hereafter referred to as "soft-bodied" invertebrates) were exposed to TFM in static or flow-through tests. Of the eight groups, tubificids (Tubifex tubifex) were the most sensitive and flatworms ranked next (the 96-h LC50's were 2.50 and 11.6 mg/l, respectively, Table 1). The sensitivity of these organisms may be greater in laboratory tests than in their natural environment. Tubificids normally live in bottom substrates, whereas those used in our test were exposed to TFM in water solutions with no substrate. Flatworms (Catenula sp.) were exposed to TFM in hard water (160 mg/l total hardness) and other organisms were exposed in soft water (20 mg/l total hardness). Because TFM is less toxic to invertebrates in hard or high pH water (Fremling 1974; Kawatski et al. 1974), the 96-h LC50 for TFM against flatworms in soft water would probably be less than 11.6 mg/l as shown in Table 1.

Organisms of intermediate sensitivity (96-h LC50's, 21.3 to 89.0 mg/l) were daphnids, seed shrimp, mayfly nymphs, and mosquito larvae. The toxicity of TFM to mayfly nymphs (Callibaetis sp. - a form that lives in streams) exposed in static and in flow-through tests did not differ significantly in either case (Table 1). The least sensitive species were glass shrimp (96-h LC50 = 125 mg/l) and backswimmers (96-h LC50 = 555 mg/l).

[1] Use of trade names does not imply U.S. Government endorsement of commercial products.

Table 1. Toxicity of TFM (39.45%) to selected aquatic invertebrates in static tests at 17 C with limed water (20 mg/l total hardness)

Species	LC50 and 95% confidence interval (mg/l) at				
	1 h	3 h	6 h	24 h	96 h
Flatworm[a]	---	44.5	35.0	25.0	11.6
Catenula sp.		30.2-66.0	27.2-45.0	18.3-34.2	9.66-14.0
Annelid	17.4	16.3	12.5	8.70	2.50
Tubifex tubifex	13.8-21.9	11.7-22.6	7.20-21.6	7.25-10.4	1.23-5.09
Daphnid	186	130	122	65.0	29.0
Daphnia magna	164-212	104-163	102-146	47.4-88.0	21.0-41.1
Seed shrimp	117	60.0	60.0	52.0	37.0
Cypridopsis sp.	80.1-171	40.0-91.1	40.0-91.1	36.2-75.0	19.0-73.2
Glass shrimp	---	660	550	215	125
Palaemonetes kadiakensis		555-785	436-694	161-286	90.6-173
Mayfly	372	182	166	100	21.3
Callibaetis sp.	214-441	157-211	135-204	60.4-166	13.8-32.7
Callibaetis sp.[b]	---	---	---	---	22.4
					15.4-32.5
Backswimmer	14,250	7,900	2,000	795	555
Notonecta sp.	9,901-20,510	5,901-10,576	1,248-3,206	718-881	472-653
Mosquito larvae	---	---	---	---	80.0
Anopheles sp.					55.5-115
Culex sp.	---	---	---	---	89.0
					66.5-119

[a] Exposed in hard water (160 mg/l total hardness).
[b] Flow-through toxicity tests.

Exposures for 96 h indicated greater toxicity than exposures for shorter periods. Invertebrates reacted differently than fish in that respect; changes in the toxicity of TFM to fish were small or nil in 3- to 96-h exposures (Marking and Olson 1975). Since TFM is applied over shorter periods (8 to 12 h) to control lamprey larvae, the values at 24 h of exposure are perhaps more important than 96-h values for estimating the sensitivity of these organisms during lampricidal treatments.

Snails and bivalves which were exposed to TFM in soft water at 17 ± 1 C, generally were more sensitive than soft-bodied invertebrates; 96-h LC50's ranged from 2 to 9 mg/l of TFM for all the species except fingernail clams (Table 2). Fingernail clams were more resistant than other mollusks, and 96-h LC50's were 16.3 and 15.3 mg/l in static and flow-through tests. TFM appeared to be more toxic to snails in static tests than in flow-through tests.

Table 2. Toxicity of TFM (39.45%) to snails, bivalves, and frog larvae in soft water (44 mg/l total hardness) at 17 ± 1 C (based on ability of organisms to respond to tactile stimulus)

Test organism	96-h LC50 and 95% confidence interval (mg/l)	
	In static tests	In flow-through tests
Snails		
Physa sp.	3.05 2.35-3.95	4.60 3.03-6.97
Helisoma sp.	3.75 3.03-4.64	4.10 2.89-5.82
Pleurocera sp.	3.90 2.96-5.14	8.65 5.51-13.6
Bivalves		
Asiatic clam *Corbicula* sp.	2.30 1.54-3.43	4.10 2.77-6.06
Mussels *Elliptio* sp.	---	3.65 2.66-5.00
Plectomerus	---	8.10 6.77-9.69
Fingernail clam *Sphaerium* sp.	16.3 10.6-25.0	15.3 7.42-31.3
Amphibians		
Gray tree frog larvae *Hyla versicolor*	1.98 1.77-2.22	---
Leopard frog larvae *Rana pipiens*	2.76 2.45-3.11	---
Bullfrog larvae *Rana catesbeiana*	---	3.55 2.62-4.82

Frog larvae also were more sensitive than soft-bodied invertebrates to TFM. In static tests larvae of the gray tree frog were the most sensitive (96-h LC50 = 1.98 mg/l), and larvae of the bullfrog were most resistant (96-h LC50 of 3.55 mg/l). Bullfrog larvae were exposed to TFM in flow-through tests and the other frog larvae in static tests. There is little difference in sensitivity among the three species.

Dawson et al. (197_) tested TFM (35.7%) for its effectiveness against larval lampreys (Petromyzon marinus) in standardized laboratory tests. In soft water (pH = 7.5) at 17 C, the 96-h LC50 was 1.60 mg/l and the 12-h LC99 was 2.90 mg/l. Thus larval lampreys were much more susceptible than the soft-bodied invertebrates.

Although the 96-h LC50 values for TFM against snails, bivalves, and frog larvae indicated sensitivity for some species at larvicidal concentrations, these organisms would be less sensitive in 12-h exposures used to treat streams for larval lampreys. Few, if any, of these organisms should be affected by stream treatments with the lampricide.

CONCLUSIONS

1. The lampricide (39.45%) was toxic to aquatic invertebrates in standardized laboratory tests, but the invertebrates were not as susceptible as larval sea lampreys under similar test conditions.
2. Most of the soft-bodied invertebrates were less sensitive than snails and bivalve mollusks to TFM.
3. Larvae of gray tree frogs were the most sensitive to TFM (96-h LC50 = 1.98 mg/l), and backswimmers were the most resistant (96-h LC50 = 555 mg/l).
4. The toxicity of TFM to invertebrates increased in longer exposures (up to 96 h), whereas the reported toxicity of TFM to fish changes little after 3-h exposures.
5. TFM appeared to be more toxic to snails in static than in flow-through tests.

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